## Xenopus Laevis as a Model System for Evaluating the Function of Fanconi Anemia Proteins

Stone, S.N.

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# Chapter 7

## Summary

Fanconi anemia (FA) is a rare autosomal and X-linked recessive disorder in which patients develop life-threatening bone marrow failure, myelodysplasia, and acute nonlymphocytic leukemia. Patients that survive to adulthood typically develop early onset epithelial cancers. Because of diverse phenotypes, FA patients are often definitively diagnosed based on their unique cytogenetic sensitivity to DNA interstrand crosslinking agents such as mitomycin C. Classical clinical features of this disease vary but often include: slowed growth, small head size, *café-au-lait* spots, and radial ray defects. FA is caused by a defect in any one out of at least 13 genes that encode proteins, which interact in a common pathway termed the "FA pathway." The FA pathway of proteins function by largely unknown mechanisms, to protect against chromosomal instability and are required for error-free DNA replication.

In Chapter 1.1 of this thesis, we provide a brief overview of the human syndromes that are associated with genomic instability and cancer predisposition.

Chapter 1.2 briefly summarizes what is currently known about the focus of this thesis, the genomic instability syndrome FA.

In Chapter 1.3 we introduce and describe the various methodologies of *Xenopus laevis* cell-free egg extracts and how such extracts may be used for the study of FA.

In Chapter 2 we provide evidence that chromatin recruitment of the FA proteins is dependent on replication and their recruitment increases when moving replication forks encounter certain DNA lesions, thus making FA proteins crucial to prevent chromosomal DNA breaks during normal replication.

In Chapter 3 we investigated the specific DNA lesions that activate the FA pathway. In a replication-free context, we found that the substrate specificity of the activated form of FANCD2 is rather broad including both single- and double-strand, linear or branched DNA lesions—but not simple DNA double strand breaks. The data in this Chapter suggests a model in which FANCD2 activation occurs during DNA replication when double stranded DNA is exposed at the replication fork due to either stalling or damage.

In Chapter 4 we introduce a novel cell-free assay using *Xenopus* extracts as an approach to identify small molecules that suppress the activation of the FA pathway, by monitoring the DNA substrate stimulated monoubiquitylation of FANCD2. We also present a novel inhibitor of FANCD2 monoubiquitylation. This Chapter further

discusses the use of the cell-free assay to re-sensitize non-FA tumor cells that have become resistant to chemotherapeutic DNA interstrand crosslinking agents such as cisplatin and melphalan.

In Chapter 5 we introduce *Xenopus laevis* as a developmental model for FA and show for the first time, the protein expression of the FA proteins during embryonic development. In addition we present evidence that *FANCG* is zygotically regulated during embryonic development.

In a general discussion we provide evidence for a biphasic recruitment of FA proteins to chromatin. During normal replication FA proteins are recruited in the absence of any exogenous damage and without checkpoint activation. After DNA damage, there is an increased recruitment of FA proteins that is ATR dependent and may be in response to specific DNA lesions.

In conclusion, the research presented in this thesis introduces a new system for dissecting the function of the FA proteins within the network of other proteins that collaborate to ensure genomic stability in vertebrates.